

61312.us.sequences.ST25  
SEQUENCE LISTING

<110> Windisch, Jorg  
Schoergendorfer, Kurt  
Palma, Norbert  
Knauseder, Franz  
Boehling, Hans

<120> Expression vectors, transformed host cells and fermentation process for the production of recombinant polypeptides

<130> BP/G-33314 LNG 61312.US

<150> PCT/EP2004/009067  
<151> 2004-08-12

<150> US 60/494,914  
<151> 2003-08-13

<160> 19

<170> PatentIn version 3.3

<210> 1  
<211> 495  
<212> DNA  
<213> Artificial

<220>  
<223> DNA encoding human interferon alpha 2B with altered codon usage

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atgcggcgaa tctctctttt ctcttgctta aaggatcgac atgacttcgg tttcccgag 120  
gaggagttcg gtaaccagtt ccaaaaggct gaaaccatcc cggtattgca tgagatgatc 180  
cagcagatct tcaacctgtt cagcactaag gactcttctg ctgcttgga tgagaccctg 240  
cttgacaaat tctacactga actgtaccag cagctgaacg acctggaagc ctgcgtgatc 300  
caggggtgtgg gtgtgactga gactccgctg atgaaggagg actctattct ggctgtgcga 360  
aaatacttcc aacggatcac tctgtatctg aaagagaaga aatacagccc gtgcgcctgg 420  
gaggttgtcc gagcagaaat catgcggtct ttctctttgt ctaccaactt gcaagaatct 480  
ttacgaagca aggaa 495

<210> 2  
<211> 27  
<212> PRT  
<213> Pseudomonas diminuta

<400> 2

Met Leu Arg Val Leu His Arg Ala Ala Ser Ala Leu Val Met Ala Thr  
1 5 10 15

Val Ile Gly Leu Ala Pro Ala Val Ala Phe Ala  
Page 1

<210> 3  
 <211> 81  
 <212> DNA  
 <213> *Pseudomonas diminuta*  
  
 <400> 3  
 atgctgagag ttctgcaccg ggcggcgctcc gccttggtta tggcgactgt gatcggcctt 60  
 gcgcccgcgg tcgcctttgc g 81  
  
 <210> 4  
 <211> 81  
 <212> DNA  
 <213> Artificial  
  
 <220>  
 <223> DNA encoding signal sequence of gac gene of *Pseudomonas diminuta*  
 with altered codon usage  
  
 <400> 4  
 atgctgagag ttctgcaccg ggcggcgctcc gccttggtta tggcgactgt gatcggcctt 60  
 gcgcccgcgg tcgcctttgc g 81  
  
 <210> 5  
 <211> 100  
 <212> DNA  
 <213> *Pseudomonas diminuta*  
  
 <400> 5  
 atcctgggttc gtacgcgccg cctacaagtg gtgatctagg ggaacgttcc gggggcgtcg 60  
 ctgcaacggc gtctccggat ctgggtgaga ggggaaatcc 100  
  
 <210> 6  
 <211> 209  
 <212> DNA  
 <213> *Pseudomonas diminuta*  
  
 <400> 6  
 tctagaccaa caacatcttc aacgtctacc cgaccaagat tcaggagccg tcggccgacc 60  
 tgggcaatgg gatgtacagc gggcttgccg cgttcggctt caccggcgga tcctggttcg 120  
 tacgcgccgc ctacaagtgg tgatctaggg gaacgttccg ggggcgtcgc tgcaacggcg 180  
 tctccggatc tgggtgagag gggaaatcc 209  
  
 <210> 7  
 <211> 23  
 <212> DNA  
 <213> Artificial  
  
 <220>  
 <223> oligonucleotide, PCR primer

<400> 7  
 taactgtcag accaagttta ctc 23

<210> 8  
 <211> 20  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Oligonucleotide, PCR primer

<400> 8  
 gcgtttcggg gatgacgggtg 20

<210> 9  
 <211> 23  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Oligonucleotide, PCR primer

<400> 9  
 tcatgtttga cagcttatca tcg 23

<210> 10  
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<220>  
 <223> Oligonucleotide, PCR primer

<400> 10  
 gggtcgagggtg gcccggtc 19

<210> 11  
 <211> 36  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Oligonucleotide, PCR primer

<400> 11  
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<210> 12  
 <211> 32  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Oligonucleotide, PCR primer

<400> 12  
 ccccccgaat tcactagtag gcgtctctct cc 32

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<210> 13  
 <211> 315  
 <212> DNA  
 <213> Artificial

<220>  
 <223> DNA comprising part of gac gene of *Pseudomonas diminuta*

<400> 13  
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 ccgacctggg caatgggatg tacagcgggc ttgcgccgtt cggcttcacc ggcggatcct 120  
 ggttcgtacg cgccgcctac aagtgggtgat ctagggggaac gttccggggg cgtcgtgca 180  
 acggcgtctc cggatctggg tgagagggga aatccatgct gagagttctg caccgggcgg 240  
 cgtccgcctt ggttatggcg actgtgatcg gccttgcgcc cgcggagaga gacgcgtact 300  
 agtgaattcg ggggg 315

<210> 14  
 <211> 11  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Oligonucleotide, part of PCR primer

<400> 14  
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<210> 15  
 <211> 23  
 <212> DNA  
 <213> Artificial

<220>  
 <223> Oligonucleotide, PCR primer

<400> 15  
 ttgcgccgc ggtcgccttt gcg 23

<210> 16  
 <211> 4  
 <212> PRT  
 <213> *Pseudomonas diminuta*

<400> 16  
 val Ala Phe Ala  
 1

<210> 17  
 <211> 540  
 <212> DNA  
 <213> Artificial

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&lt;220&gt;

&lt;223&gt; DNA comprising nucleotide sequence encoding human interferon alpha 2B

&lt;400&gt; 17

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ggggggccgc ggtcgccttt gcgtgcgac tgccgcaaac ccacagcctg ggtagccggc      60
gaaccttgat gcttctggca cagatgcgyc gaatctctct tttctcttgc ttaaaggatc    120
gacatgactt cggtttcccg caggaggagt tcggtaacca gttccaaaag gctgaaacca    180
tcccgggtatt gcatgagatg atccagcaga tcttcaacct gttcagcact aaggactctt    240
ctgctgcttg ggatgagacc ctgcttgaca aattctacac tgaactgtac cagcagctga    300
acgacctgga agcctgcgtg atccagggtg tgggtgtgac tgagactccg ctgatgaagg    360
aggactctat tctggctgtg cgaaaatact tccaacggat cactctgtat ctgaaagaga    420
agaaatacag cccgtgcgcc tgggaggttg tccgagcaga aatcatgcgg tctttctctt    480
tgtctaccaa cttgcaagaa tctttacgaa gcaaggaata atacgcgtga attcgggggg    540

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&lt;210&gt; 18

&lt;211&gt; 807

&lt;212&gt; DNA

&lt;213&gt; Artificial

&lt;220&gt;

&lt;223&gt; DNA encoding fusion protein comprising signal sequence of gac gene of Pseudomonas diminuta and human interferon alpha 2B

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (210)..(788)

&lt;400&gt; 18

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tctagaccaa caacatcttc aacgtctacc cgaccaagat tcaggagccg tcggccgacc      60
tgggcaatgg gatgtacagc gggcttgccg cgttcggctt caccggcgga tcctggttcg    120
tacgcgccgc ctacaagtgg tgatctaggg gaacgttccg ggggcgtcgc tgcaacggcg    180
tctccggatc tgggtgagag gggaaatcc atg ctg aga gtt ctg cac cgg gcg      233
                               Met Leu Arg Val Leu His Arg Ala
                               1      5
gcg tcc gcc ttg gtt atg gcg act gtg atc ggc ctt gcg ccc gcg gtc      281
Ala Ser Ala Leu Val Met Ala Thr Val Ile Gly Leu Ala Pro Ala Val
10      15      20
gcc ttt gcg tgc gat ctg ccg caa acc cac agc ctg ggt agc cgg cga      329
Ala Phe Ala Cys Asp Leu Pro Gln Thr His Ser Leu Gly Ser Arg Arg
25      30      35      40
acc ttg atg ctt ctg gca cag atg cgg cga atc tct ctt ttc tct tgc      377
Thr Leu Met Leu Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys
45      50      55
tta aag gat cga cat gac ttc ggt ttc ccg cag gag gag ttc ggt aac      425
Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Gly Asn

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60	65	70	
cag ttc caa aag gct gaa acc atc ccg gta ttg cat gag atg atc cag Gln Phe Gln Lys Ala Glu Thr Ile Pro Val Leu His Glu Met Ile Gln 75 80 85			473
cag atc ttc aac ctg ttc agc act aag gac tct tct gct gct tgg gat Gln Ile Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Ala Trp Asp 90 95 100			521
gag acc ctg ctt gac aaa ttc tac act gaa ctg tac cag cag ctg aac Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn 105 110 115 120			569
gac ctg gaa gcc tgc gtg atc cag ggt gtg ggt gtg act gag act ccg Asp Leu Glu Ala Cys Val Ile Gln Gly Val Gly Val Thr Glu Thr Pro 125 130 135			617
ctg atg aag gag gac tct att ctg gct gtg cga aaa tac ttc caa cgg Leu Met Lys Glu Asp Ser Ile Leu Ala Val Arg Lys Tyr Phe Gln Arg 140 145 150			665
atc act ctg tat ctg aaa gag aag aaa tac agc ccg tgc gcc tgg gag Ile Thr Leu Tyr Leu Lys Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu 155 160 165			713
gtt gtc cga gca gaa atc atg cgg tct ttc tct ttg tct acc aac ttg Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Thr Asn Leu 170 175 180			761
caa gaa tct tta cga agc aag gaa taa tacgcgtact agtgaattc Gln Glu Ser Leu Arg Ser Lys Glu 185 190			807

<210> 19  
 <211> 192  
 <212> PRT  
 <213> Artificial

<220>  
 <223> Synthetic Construct

<400> 19

Met Leu Arg Val Leu His Arg Ala Ala Ser Ala Leu Val Met Ala Thr  
 1 5 10 15

Val Ile Gly Leu Ala Pro Ala Val Ala Phe Ala Cys Asp Leu Pro Gln  
 20 25 30

Thr His Ser Leu Gly Ser Arg Arg Thr Leu Met Leu Leu Ala Gln Met  
 35 40 45

Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly  
 50 55 60

Phe Pro Gln Glu Glu Phe Gly Asn Gln Phe Gln Lys Ala Glu Thr Ile  
 65 70 75 80

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Pro Val Leu His Glu Met Ile Gln Gln Ile Phe Asn Leu Phe Ser Thr  
85 90 95

Lys Asp Ser Ser Ala Ala Trp Asp Glu Thr Leu Leu Asp Lys Phe Tyr  
100 105 110

Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Ile Gln  
115 120 125

Gly Val Gly Val Thr Glu Thr Pro Leu Met Lys Glu Asp Ser Ile Leu  
130 135 140

Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Lys Glu Lys  
145 150 155 160

Lys Tyr Ser Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg  
165 170 175

Ser Phe Ser Leu Ser Thr Asn Leu Gln Glu Ser Leu Arg Ser Lys Glu  
180 185 190